Comparison of Phytase Production by Aspergillus Ficuum under Submerged and Solid State Fermentation Conditions

M Badamchi[1], Z Hamidi-Esfahani[2], S Abbasi[3]

[1,2,3] Department. of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

[1]Mahsabadamchi@yahoo.com; [2]Hamidy_z@modares.ac.ir; [3]sabassifood@modares.ac.ir

Abstract

Firstly, improvement of culture condition on phytase production by *Aspergillus ficuum* was investigated using submerged fermentation. The four factors studied were significantly effective on the phytase production. The optimum values for the factors were determined via response surface methodology (RSM) as: glucose, 5.23 g/100 mL; ammonium sulphate, 1.6 g/100 mL; wheat bran, 3.28 g/100 mL, and fermentation time, 198.30 h. The produced amount of phytase under these conditions was 40.21 U/ mL. In the next stage, the process was scaled up to a 3-L batch bioreactor. The highest level of phytase produced in this stage was 85.41 U/mL, which resulted in a 2.1-fold enhancement. In the last stage, the results of optimization of phytase production in solid-state fermentation were further scaled up to packed bed solid-state bioreactor. The highest level of phytase produced in this stage was 87.75 U/gds, which resulted in a 3.4-fold enhancement.

Keywords

Phytase; Aspergillus Ficuum; Packed Bed Solid-State Bioreactor; Batch Bioreactor

Introduction

Phytases (EC 3.1.3.8 and EC 3.1.3.26) belong to the family of histidine acid phosphatases which catalyze the hydrolytic degradation of phytic acid and its salts (phytates), generally yielding inositol, inositol monophosphate, and inorganic phosphate (Mullaney *et al.* 2000). Phytate (myo-inositol hexakisphosphate) is the primary storage form of phosphorus and inositol in plant seeds and grains, and serves as a major source of nutrients for animals. However, the bound phosphorus in phytate is poorly utilized in digestive tract of monogastric animals (Fugthong *et al.* 2010). Supplementation of diets with inorganic phosphorus along with the excreted phytate phosphorus, however, imposes global ecological problems when phosphorus enters into rivers, resulting in cyanobacterial blooms, hypoxia, and death of marine animals (Naqvi *et al.* 2000). Although several strains of bacteria, yeasts, and fungi have been used for production of phytase, two strains of *Aspergillus sp*., *A. niger* and *A. ficuum*, have most commonly been employed for commercial production (Sabu *et al.* 2005). The market volume of phytases is in the range of € 150 million, which is still rising further (Singh *et al.* 2011).

Depending on the culture conditions and the genotype of the strain, the growth form of the filamentous fungi in submerged fermentation (SmF) varies between two extremes, the pelleted and the filamentous. Each of these forms has its own characteristics, which can affect the process productivity by influencing the mass transfer rates (Mitchell and Lonsane 1992). While in SmF, the fungus is exposed to hydrodynamic forces, in solid-state fermentation (SSF), growth is restricted to the surface of the solid matrix. The growth characteristics in such systems depend on the availability of nutrients and the geometric configuration of the matrix (Papagianni *et al.* 1999).

The present investigation has evaluated the effect of different nutritional ingredients, such as carbon, nitrogen, and phosphate; and the fermentation time on the production of phytase enzyme by *Aspergillus ficuum* PTCC 5288 under submerged conditions. Then, the conditions were optimized to achieve maximum production in batch bioreactor. Finally, the results were compared with production of phytase in packedbed solid-state bioreactor.

Materials and Methods

Materials

Wheat bran was supplied by the flour mill (Morshedy Company, Tehran Province, Iran). Phytic acid sodium salt was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade and

obtained from leading manufacturers including Merck and Sigma.

Fungal Strain

The strain used in the present work was *Aspergillus ficuum* PTCC 5288. A spore suspension was obtained by growing the organism on potato dextrose agar at 24°C for two weeks. The spores were harvested with known quantities of water containing 0.1% Tween-80, and then the concentrations were adjusted to 5×10^6 and 3×10^7 spores/mL for SSF and SmF inoculations, respectively. After addition of 23% glycerol as the cryoprotective agent, the spore suspension obtained was frozen at -80°C.

Medium and Culture Conditions

1）Submerged Fermentation in Shake Flasks

Each 100 mL of the submerged fermentation medium contained KCl, 0.05 g; $MgSO_4.7H_2O$, 0.05 g; $FeSO_4.7H_2O$, 0.01 g; wheat bran, variable; glucose, variable; and $(NH_4)_2SO_4$, variable. The media, 100 mL in a 250-mL shake flask, was sterilized at 120°C for 20 min. The fermentations medium was then allowed to cool, inoculated with 1% (v/v) spore suspension, and incubated at 30°C at 180 rpm. The experiments were conducted according to the statistical design. Variations in the process parameters (the amount of wheat bran, glucose, and ammonium sulphate; and the duration of incubating time) were also maintained according to the statistical design. Fermentation processes were carried out in duplicate.

Batch Bioreactor

After optimizing the production of phytase under shake flasks conditions, it was further optimized in batch bioreactor (Majer Science-F_1-S-3L, Taiwan) with working volume of 1 L. The medium and production conditions were adopted on the basis of studies carried out in the shake flasks level. The fermenter was run with aeration of 1, 1.5, 2 vvm and agitation rate of 180 rpm. Inoculum was prepared by inoculating 250-mL shake flask containing the fermentation medium inoculated with 3×10^7 spores/mL, and incubated at 30°C at 200 rpm for 72 h. The fermenter containing 950 mL of the medium was sterilized and then inoculated automatically. The fermenter was equipped with different controls such as pH, temperature, dissolved oxygen, agitation, and antifoam.

Packed Bed Solid-State Bioreactor

The SSF process was carried out in glass column bioreactors (equipped with the heating jackets) (the size of interior column, which was filled with the medium was 80 × 4 cm) using 0.6–2.0-mm particles of wheat bran (Fig 1). The medium consisted of wheat bran, 5 g; glucose, 10.14%; $(NH_4)_2SO_4$, 4%; and $MgSO_4$, 0.64% (Jafari-Tapeh 2008). The initial moisture content of the media was adjusted to 63%. Inoculum was prepared by inoculating a 250-mL shake flask containing fermentation medium with 5×10^6 spores/mL and incubating the flask at 30°C for 72 h. The fermenter, containing 60 g of the medium (per column), was sterilized and then inoculated aseptically. The inoculum for SSF was 10% v/v of medium and added to each column. The aeration volumetric rate varied from 0.1 to 0.5 vvm. For better air distribution, 1/3 of the column volume was left free, and only two third of it was filled. These columns were connected to humidifiers and water bath at 30°C for 119.23 h. Fermentation processes were carried out in duplicate.

Extraction of Crude Phytase in SmF

Of the cell culture, 45 mL was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant obtained was stored at 4°C and used as crude phytase enzyme.

Extraction of Crude Phytase in SSF

Crude phytase was extracted by mixing the moldy bran with 1:5 w/v of acetate buffer (0.2 M) at pH 5.5 in a rotary shaker for 20 min at 200 rpm. The aqueous solution was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used as crude enzyme preparation for further investigation.

Phytase Assay

Crude enzyme extracted from both medium (SmF and SSF) was quantitatively assayed for phytase enzyme. The assay was initiated by mixing 1 mL of the diluted crude enzyme (1:10) with 0.5 mL of sodium acetate (0.2 M) buffer of pH 4.5 and 0.5 mL of sodium phytate (15 mM) (Sigma Chemicals Co, USA).

The reaction mixture was incubated at 40°C in a water bath for 45 min. The reaction was terminated by adding 2 mL of 15% trichloro acetic acid. Then, 0.5 mL of the assay mixture was mixed with 4 mL of 2:1:1 v/v acetone, 10 mM ammonium molybdate, and 5 N sulfuric acid (AAM solution); and 0.4 mL of citric acid (1 M). The amount of free phosphate released was determined by spectrophotometer at 355 nm. A standard graph was plotted using potassium dihydrogen phosphate with working concentration ranging from 30 to 360 μM (Heinonen and Lahti 1999).

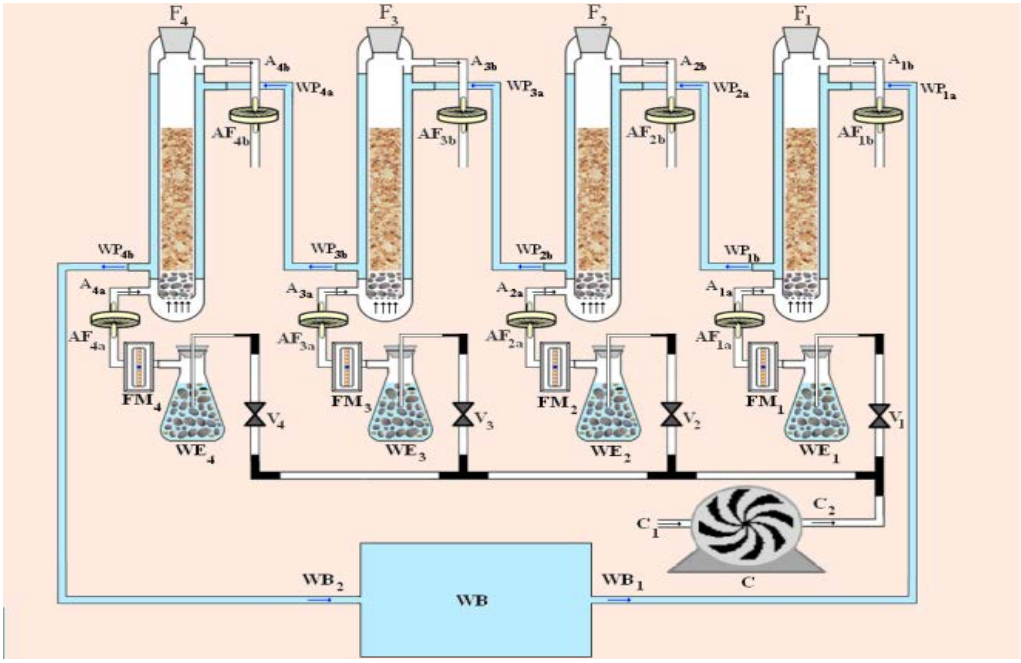

FIG 1 THE PACKED BED SOLID-STATE BIOREACTOR LEGEND: BIOREACTOR FLASKS (F_1, F_2, F_3, F_4), AIR ENTRANCES TO THE BIOREACTORS (A_{1A}, A_{2A},A_{3A}, A_{4A}), EXIT PORTS OF EXHAUSTED GASES FROM THE BIOREACTORS (A_{1B}, A_{2B},A_{3B}, A_{4B}), FILTERS OF AIR ENTRANCES TO THE BIOREACTORS (AF_{1A}, AF_{2A},AF_{3A}, AF_{4A}), FILTERS FOR EXHAUSTED GASES AFA_2, PUMP C (C_1 AIR ENTRANCE, C_2 AIR EXIT), VALVE FOR ADJUSTMENT OF AIR RATE (V_1, V_2, V_3, V_4),HUMIDIFIERS (WE_1, WE_2, WE_3, WE_4), FLOW METERS (FM_1, FM_2, FM_3, FM_4), WATER BATH ROOM CIRCULATED WB (WB_1 WATER ENTRANCE, WATER EXIT WB_2), WATER ENTRANCES TO THE BIOREACTORS (WP_{1A}, WP_{2A},WP_{3A}, WP_{4A}), WATER EXIT PORTSFROM THE BIOREACTORS (WP_{1B}, WP_{2B},WP_{3B}, WP_{4B})

Optimization of Critical Variables Using Response Surface Meyhodology

The critical variables of wheat bran, glucose, ammonium sulphate, and incubation time were further optimized by RSM in the synthetic medium [g/100 mL: KCl, 0.05 g; $MgSO_4.7H_2O$, 0.05 g; $FeSO_4.7H_2O$, 0.01 g; wheat bran, variable; glucose, variable; and $(NH_4)_2SO_4$, variable] by central composite design (CCD) to find out their optimal values and to study their interactions. The effects of each independent variable were studied at five different levels (-α, -1, 0, +1, and +α; Table 1) and a set of 28 runs were conducted.

TABLE 1 EXPERIMENTAL VARIABLES AT DIFFERENT LEVELS USED FOR RSM APPROACH

Variables	Units	Symbol code	Levels				
			-α	-1	0	+1	+α
Glucose	%(g/100mL)	A	2	3.5	5	6.5	5
Ammonium sulphate	%(g/100mL)	B	1	2	3	4	5
wheat bran	%(g/100mL)	C	1	2	3	4	5
Incubation period	Hour	D	48	120	192	264	336

All the variables were taken at a central coded value of zero. The minimum and maximum ranges of the variables were used and the full experimental plan with regard to their values in coded form is provided in Table 2. The response value (Y) in each trial was the average of the duplicates. The experimental results of the CCD were fitted with a second-order polynomial equation by multiple regression procedure. Behavior of the system was explained by the following quadratic equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{23} BC + \beta_{24} BD + \beta_{34} CD + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2$$

Where Y is the predicted response, β_0 is the intercept, β_1, β_2, β_3, and β_4 are linear coefficients, β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, and β_{34} are interaction coefficients, β_{11}, β_{22}, β_{33}, and β_{44} are squared coefficient, and *A*, *B*, and C are independent variables. The quality of fit of the second-order model equation was expressed by the coefficient of determination (R^2), and its statistical significance was determined by an F-test. Significance of each regression coefficient was verified by a t-test.

Result and Discussion

Optimization of Submerged Fermentation in Shake Flasks by Response Surface Meyhodology

RSM is a widely used statistical method based on the multivariate non-linear model for optimization of

fermentation media (Gu *et al.*2005; Cui *et al.*2006). Considering the interactions of various parameters during the fermentation process, RSM examines the responses of several factors by varying them simultaneously with a limited number of experiments (Kalil *et al.*2000; Mundra *et al.* 2007). To obtain the maximum phytase yield, RSM was applied to determine the optimal variables. Interactions among the factors were estimated to reach an empirical model related with the real values. In RSM, each selected variable (carbon source (glucose), nitrogen source (ammonium sulphate), phosphorus source (wheat bran), and fermentation time) was studied at five different levels along with other variables. In this way, the interactions among the variables at different levels could be studied. The predicted values of phytase production along with the experimental data are given in Table 2. The observations revealed a variation from 19.64 to 70.70 U/mL for phytase production, reflecting the importance of medium optimization to attain higher productivity. The experimental data were fitted to a second-order polynomial equation, and the regression equation coefficients were calculated. The response (phytase activity) can be expressed as a function of the values of glucose (*A*), ammonium sulphate (*B*), wheat bran (*C*), and incubation time (*D*):

$$Y=34.46+2.99A-4.88B-2.70C+5.72D-2.59AB+2.17AD-2.58BC-3.12BD-1.43CD-1.72A^2-1.67B^2+2.03D^2$$

TABLE 2 EXPERIMENTAL DESIGN AND RESULTS OF CCD OF RESPONSE SURFACE METHODOLOGY

RunNo	A Glucose (%)	B Ammonium sulphate (%)	C wheat bran (%)	D Incubation period (Hour)	Phytase activity (U/mL)		
					Experimental 1	Experimental 2	Predicted
1	-1	-1	-1	-1	28.48	28.91	27.43
2	-1	-1	-1	1	42.42	46.11	43.63
3	-1	-1	1	-1	30.65	31.92	30.58
4	-1	-1	1	1	43.86	36.58	41.08
5	-1	1	-1	-1	29.52	31.26	34.26
6	-1	1	-1	1	39.91	39.47	37.96
7	-1	1	1	-1	29.64	23.76	27.08
8	-1	1	1	1	28.44	24.37	25.09
9	1	-1	-1	-1	32.66	35.94	34.80
10	1	-1	-1	1	48.44	70.70	59.67
11	1	-1	1	-1	35.55	33.76	36.87
12	1	-1	1	1	58.74	62.70	56.04
13	1	1	-1	-1	31.20	32.06	31.26
14	1	1	-1	1	40.38	47.12	43.63
15	1	1	1	-1	24.43	22.05	23.01
16	1	1	1	1	28.62	27.27	29.68
17	$-\alpha$	0	0	0	37.60	33.61	35.68
18	α	0	0	0	47.93	46.84	47.65
19	0	$-\alpha$	0	0	37.58	35.02	37.91
20	0	α	0	0	19.64	19.64	18.37
21	0	0	$-\alpha$	0	37.84	39.25	38.18
22	0	0	α	0	27.07	26.29	27.38
23	0	0	0	$-\alpha$	32.31	35.41	31.50
24	0	0	0	α	50.21	53.13	54.37
25	0	0	0	0	34.95	36.56	35.47
26	0	0	0	0	35.80	34.56	35.47
27	0	0	0	0	39.84	38.71	35.47
28	0	0	0	0	25.17	38.16	35.47

TABLE 3.ANALYSIS OF VARIANCE (ANOVA) FOR THE QUADRATIC MODEL

Source	Sum of squares	df	F Value	Prob> F
Model	5064.61	12	22.49	< 0.0001
A	429.44	1	26.69	< 0.0001
B	1145.09	1	71.17	< 0.0001
C	349.93	1	21.75	< 0.0001
D	1569.29	1	97.54	< 0.0001
AB	214.96	1	13.36	0.0007
AD	150.55	1	9.36	0.0036
BC	213.02	1	13.24	0.0007
BD	312.10	1	19.40	< 0.0001
CD	64.99	1	4.04	0.0494
A2	150.91	1	7.16	0.0036
B2	141.99	1	10.03	0.0064
C2	211.93	1	10.39	0.0007
Residual	683.64	43		
Lack of fit	166.05	12	0.83	0.6213
Pure error	517.59	31		
Total	5724.25	55		

Table 3 presents the p-value and F-value. The statistical analysis for the model (Table 3) showed that the ''lack of fit'' was not statistically significant (p= 0.6213>0.05). The p value for the model was less than 0.0001, indicating that the model was significant and could be used in monitoring the optimization. Quality of the model could be checked using various criteria. The coefficient of determination (adjusted R^2) was 0.8472 for phytase production, which explained 84.72% variability in the model. The R^2 value should lie in between 0 and 1. The closer the R^2 value is to be 1.0, the stronger the model is, and the better it predicts the response (Haaland, 1989).The correlation coefficient value (predicted R^2) for phytase production was 0.8806, which indicates a strong correlation between the experimental and predicted values of phytase production. Suggesting values of ''Prob> F'' less than 0.05 indicated that the model terms were significant. *A, B, C, D, AB, AD, BC, BD,* A^2, B^2, and D^2 were significant terms of the model for phytase production. In order to determine the levels of each variable for maximum phytase production, three-dimensional response surface plots were constructed by plotting the response (phytase production) on the z-axis against any two independent variables, while other variables were kept constant at the level of zero (Figs. 2-5). The shape of the response surface curves showed a moderate interaction between the variables tested.

Validation of the Model

In order to obtain the maximum activity of phytase, optimization of the model was performed using RSM auto-analysis software (Design Expert, version 7.0.0.1) by setting the maximum phytase activity value (Y) as the goal. The resulting maximum phytase activity value (Y) of 39.61 U/mL was predicted under the following optimal conditions: glucose, 5.23 g/100 mL, ammonium sulphate, 1.6 g/100 mL; wheat bran, 3.28 g/100 mL; and the fermentation time, 198.30 h. The model was validated by repeating the experiments under the optimized conditions, which resulted in the phytase production of 40.21 U/mL (predicted response: 39.61 U/mL) indicating the efficacy of the model for prediction of the amount of phytase production under different medium conditions.Phytase activity reported during present work in eight days of submerged fermentation was very high, i.e., 40.21 U/mL as compared to 11 IU/mL by *Aspergillusniger van Teigham* after 17 days of submerged fermentation and 6.6 IU/mL by *Aspergillusficuum* NRRL 3135 (Vats *et al.* 2004; Wodzinski and Ullah 1996).

Effect of Variables on Phytase Production

1) Interaction of Ammonium Sulphate and Glucose

The contour and three-dimensional plots of interactions among the variables showed an increase in phytase production as the concentration of glucose increased and the concentration of ammonium sulphate increased up to 4%, after that enzyme production declined (Fig. 2). The effect of nitrogen sources on enzyme production by fungi is complicated. It is generally accepted that inorganic

nitrogen sources are more easily assimilated than organic nitrogen sources by fungi. This may be due to repression of enzyme activity at high nitrogen. Addition of easily metabolizable sugars may help the organism to generate rapid biomass. Easily metabolizable sugar e.g., glucose has been reported to increase phytase production by *Aspergillus niger* in submerged and solid-state fermentation (Vats and Banerjee 2004; Vats and Banerjee2002; Vats *et al* 2004).

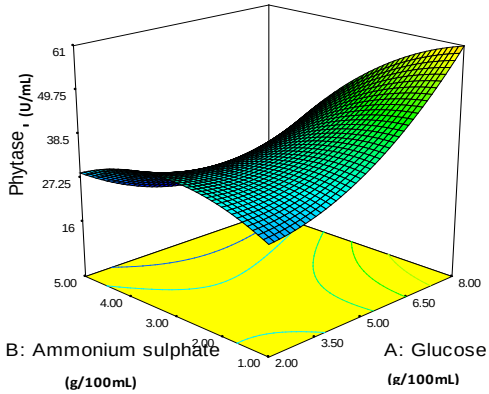

FIG..1 EFFECT OF THE INTERACTION OF AMMONIUM SULPHATE AND GLUCOSE ON PHYTASE PRODUCTION WHILE KEEPING THE CODED VALUES OF WHEAT BRAN AND INCUBATION PERIOD AT ZERO LEVEL

2) Interaction of Glucose and Incubation Period

The contour and three-dimensional plots of interactions among the variables showed an increase in phytase production as the level of glucose (from 2% up to 8%) and duration of incubation period (up to 14 day) increased (Fig. 3). Soni and Khire reported increase in phytase activity up to 15 day of submerged fermentation with *Aspergillus niger* NCIM 563. Moreover, Vohra and Satyanarayana showed high phytase activity with Pichiaanomala, when the medium contained 40 g/l of glucose (Soni and Khire2007 ;Vohra and Satyanarayana 2002).

3) Interaction of Wheat Bran and Ammonium Sulphate

The contour and three-dimensional plots of interactions among the variables showed a decrease in phytase production as the level of wheat bran and ammonium sulphate increased (Fig. 4). As far as the economic and environmental is concerned, utilization of agricultural residues is getting more attention. Wheat bran also contains phytic acid, an inducer of phytase. It has been reported that phytase synthesis is repressed by excessive amount of orthophosphate, and the extent of repression depending on the strain and that repression can be reversed by providing a slow-release organic phosphate source (Papagianni *et al.* 1999). From this point of view, the inclusion of wheat bran is beneficial.

Singhand Satyanarayana achieved a slight enhancement in phytase production by a thermophilic mould *Sporotrichum thermophile* in submerged fermentation, 3% more than that achieved by the wheat bran. However, as the amount of mould increased, the fermentation began to resemble semi-solid-state fermentation (Singh and Satyanarayana, 2008).

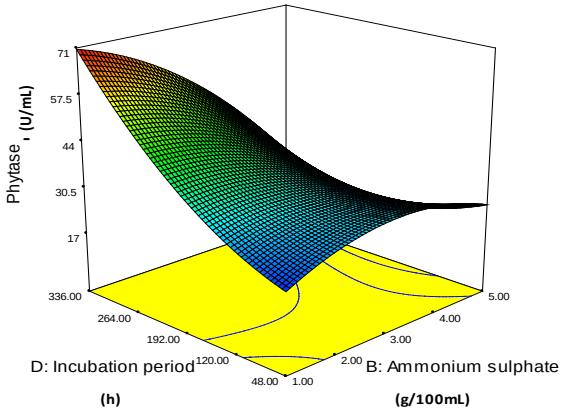

FIG. 3 EFFECT OF THE INTERACTION OF INCUBATION PERIOD AND GLUCOSE ON PHYTASE PRODUCTION WHILE KEEPING CODED VALUES OF AMMONIUM SULPHATE AND WHEAT BRAN AT ZERO LEVEL

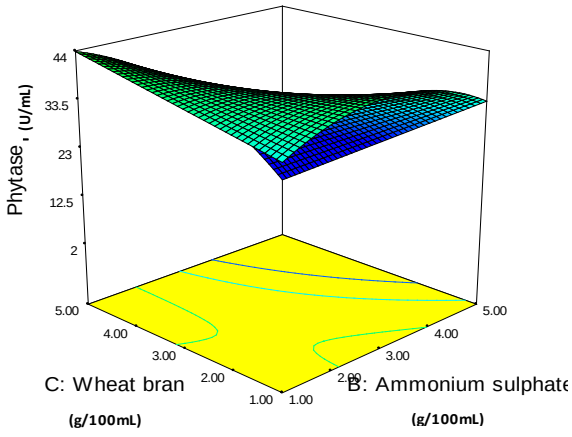

FIG. 4 EFFECT OF THE INTERACTION OF WHEAT BRAN AND AMMONIUM SULPHATE ON PHYTASE PRODUCTION WHILE KEEPING CODED VALUES OF GLUCOSE AND INCUBATION PERIOD AT ZERO LEVEL

4) Interaction of Wheat Bran and Ammonium Sulphate

The contour and three-dimensional plots of interactions among the variables showed an increase in phytase production as the incubation period increased and the concentration of ammonium sulphate decreased (Fig. 5). At the beginning of the fermentation process, increasing the amount of ammonium sulphate increased phytase production.

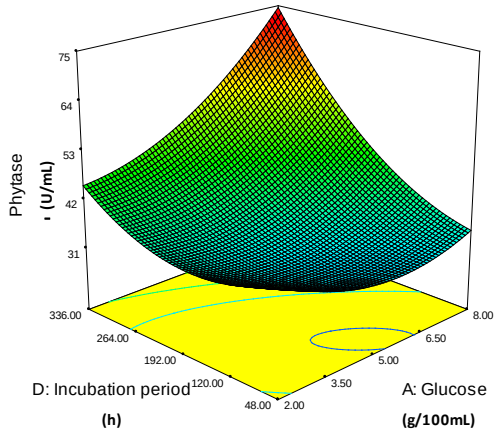

FIG. 5 EFFECT OF THE INTERACTION OF INCUBATION PERIOD AND AMMONIUM SULPHATE ON PHYTASE PRODUCTION WHILE KEEPING CODED VALUES OF GLUCOSE AND WHEAT BRAN AT ZERO LEVEL

Scaling up Phytase Production (Batch Bioreactor)

The production pattern of phytase by A. ficuum PTCC 5288 was studied using a laboratory scale fermenter (The Majer Science-F1-S-3L, Taiwan) of 3-L capacity with a working volume of 1 L. The fermenter was run with aeration of 1, 1.5, and 2 vvm; and agitation rate of 180 rpm. The aeration rate showed significant effect on phytase production (Table 4). This may be due to requirement of microaerophilic atmosphere for A. ficuum PTCC 5288 to secrete higher amounts of phytase. The highest amount of phytase produced in this stage was 85.41 U/mL, which was 2.1-fold higher than that in shake flasks.However, in case of Aspergillusniger NCIM 563 in a 3-L fermenter, maximum phytase activities of 68 and 66 IU/mL were obtained on the 10th day of fermentation at glucose concentrations of 4 and 3.5%, respectively (Shah et al. 2009) .Furthermore, in case of *A. niger van Teighem*, phytase production in the 7-L fermenter was significantly influenced by higher agitation rates. A maximum of 12.24 IU/mL phytase was achieved at 300 rpm (Vats and Banerjee 2004).

TABLE 4 EFFECT OF AERATION RATE ON PHYTASE PRODUCTION IN TANK BIOREACTOR

Aeration rate (L/min)	Phytase activity (U/mL)	Phytase productivity (U/L/d)
0.5	54.27	6783.75
1.0	62.83	7553.75
1.5	85.41	10676.25

Scaling up Phytase Production in Packed Bed Solid-State Bioreactor

As provided in Table 5, increase in the aeration flow rate led to higher phytase production. In comparison to the process carried out without aeration, using forced air improved phytase formation. It is well known that oxygen is an important culture parameter for fungal growth. As the fungal mycelium develops on a solid surface, the void spaces between the hyphae can either be fully or partially filled with water. In the former case, it results in severe oxygen limitation, causing anaerobic conditions affecting the performance of SSF (Oostra, *et al.* 2001; Raghavarao *et al.* 2003). These results are consistent with those reported by Spier and co-workers (2009), where phytase production by *A. niger* FS3 increased with forced air and phytase synthesis was stimulated with forced air in a column-type bioreactor. The highest level of phytase produced in this stage was 86.75 U/gds.

TABLE 5 EFFECT OF AERATION RATE ON PHYTASE PRODUCTION IN PACKEDBED SOLI-STATE BIOREACTOR

Aeration rate (L/min)	Phytase activity (U/gds)	Phytase productivity (U/kg/d)
0.1	43.87± 3.0	8974
0.3	59.26± 4.0	11850
0.5	86.75± 3.0	17350

Conclusion

Four factors including carbon source (glucose), nitrogen source (ammonium sulphate), phosphorus source (wheat bran), and fermentation time had significant effect on the production of phytase. Scaling up the process to 3-L batch bioreactor resulted in a 2.1-fold enhancement in phytase production. By scaling up to packedbed solid-state bioreactor and applying aeration, the highest level of phytase produced in this stage was 87.75 U/gds, which resulted in a 3.4-fold enhancement. The phytase productivity by *Aspergillus ficuum* PTCC was higher in packed bed solid-state bioreactor (17350 U/kg/d) than that intank bioreactor (10676.25 U/L/d).

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